# Hyperthermia, antipyretics and function of polymorphonuclear leukocytes

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**Whether hyperthermia (temperature, 40°C), salicylates, acetaminophen or phenacetin has an adverse effect on polymorphonuclear leukocyte (PMNL) function was examined. Migration experiments were carried out in Boyden chambers with bacterial chemotactic factor as the attractant, and bactericidal assays were done with *Staphylococcus aureus* and serum from an AB blood group donor as a source of opsonins. PMNL viability was determined by the trypan blue exclusion method.**

**Neither hyperthermia nor any of the drugs tested affected PMNL viability adversely, but sodium salicylate and phenacetin suppressed PMNL migration. Early staphylococcal killing was greater at 40°C; however, after 2 hours the converse was true. Bactericidal activity was suppressed by acetylsalicylic acid, sodium salicylate and phenacetin.**

**Hence it appears PMNL function is similar at 37° and 40°C but that some commonly used antipyretics have an adverse effect on PMNL activity.**

**On a examiné si l'hyperthermie (une température de 40°C), les salicylates, l'acétaminophène ou la phénacétine avaient un effet adverse sur la fonction des leucocytes polymorphonucléaires (LPMN). Des expériences de migration ont été réalisées dans des chambres de Boyden utilisant un facteur de chimiotaxie bactérien comme stimulant, et des essais d'effet bactéricide ont été faits sur *Staphylococcus aureus* avec le sérum d'un donneur de type AB comme source d'opsonines. La survie des LPMN a été déterminée par la méthode d'exclusion du bleu trypan.**

**Ni l'hyperthermie ni aucun des drogues testées n'ont nui à la survie des LPMN, mais le salicylate de sodium et la phénacétine ont supprimé la migration des LPMN. Au début, la destruction du staphylocoque était plus grande à 40°C; toutefois, après 2 heures on a constaté l'inverse. L'activité bactéricide a été supprimée par l'acide acétylsalicylique, le salicylate de sodium et la phénacétine.**

**Donc, il semble que la fonction des LPMN soit semblable à 37°C et à 40°C mais que quelques uns des antipyrétiques d'usage courant aient un effet adverse sur l'activité des LPMN.**

Although hyperpyrexia is a cardinal sign of infection, its role in the morbidity of an infectious illness is unclear.[1] Studies with animals have demonstrated that the mortality following bacterial infection can be reduced by preventing fever.[2] Similar evidence for humans is lacking.[3]

Agents used to reduce temperature have potentially serious side effects, which include coagulation and acid–base disturbances with salicylates,[4] hepatotoxicity with acetaminophen[5] and nephrotoxicity ascribed to phenacetin.[6] If these drugs interfere with the host defence response they may be detrimental to the person with an infectious illness. In this paper the effect of hyperthermia (temperature, 40°C) and the antipyretics mentioned above on polymorphonuclear leukocyte (PMNL) viability and function was ascertained.

## Methods

Healthy adult donors were used as the source of PMNLs. The cells were isolated by a method previously described[7] and then suspended in Krebs–Ringer phosphate (KRP) solution, pH 7.45, to which was added 0.006 M glucose and 0.35% bovine serum albumin (Sigma Chemicals, St. Louis, Missouri).

Chemotactic attractant was prepared from the bacteria-free supernatant of an 18-hour growth of *Escherichia coli* (hospital strain) in medium 199 (Difco Laboratories, Detroit, Michigan). This was diluted to 25% by volume with KRP solution, then placed in the lower compartment of a Boyden chamber (Schleicher and Schuell, Keene, New Hampshire). Simultaneous drug-free controls were run with 25% medium 199 in KRP solution. The upper chamber containing $2.5 \times 10^6$ PMNLs was separated from the lower by a membrane filter (3-$\mu$m pore size) (Sartorius-membrane filter GMBH-34, Gottingen, West Germany). All testing was carried out in duplicate.

The chambers were incubated for 3 hours in a humidified environment; the filters were then removed, fixed, stained, mounted[8] and examined. The average number of cells that had migrated to the undersurface of the filter membrane in five random fields within the area of a Whipple micrometer disc (Canlabs Limited, Toronto) at a magnification of ×400 was determined. The average value from the control chambers representing random migration was subtracted from the stimulated value to give a final number expressed as the chemotactic migration value.

In the bactericidal experiments, a hospital strain of *Staphylococcus aureus* was grown overnight in trypticase soy yeast broth (Difco Laboratories), separated by centrifugation, washed and resuspended in 0.9% sodium chloride to approximately $5.0 \times 10^7$ colony

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forming units (CFU)/mL. To 0.1 mL of this was added 0.5 mL of PMNL suspension containing $1.0 \times 10^7$ cells, 0.1 mL of serum from an AB blood group donor as a source of opsonins, 0.1 mL of the appropriate drug dissolved in 0.9% sodium chloride, with KRP solution added to a total volume of 1.0 mL. Any shift in pH secondary to the drugs tested was corrected with 10% sodium bicarbonate. The mixture was placed in capped plastic culture tubes (Falcon, Oxnard, California) and agitated on a Hema-Tek aliquot mixer (Fisher Scientific Co. Ltd., Toronto). Control tubes lacking PMNLs were run simultaneously. A zero-time specimen was taken from the bacteria–saline suspension and 0.1-mL aliquots were removed from the culture tubes 15, 30, 60 and 120 minutes after incubation. These were diluted serially with distilled water and quantitative pour-plates were made. Results were counted after 24 hours of incubation at 37°C. All assays were done in duplicate, the final result being the average of the two values.

Chemotactic and bactericidal experiments were carried out at 37°C, with the exception of the hyperthermia experiments, which were run at 40°C. In all experiments, cells were preincubated for 60 minutes in the appropriate concentration of drug and at the appropriate temperature.

Viability testing was done with the trypan blue exclusion method.[9] Testing was carried out after 3 hours of incubation at 37° or 40°C.

Drugs tested included acetylsalicylic acid (ASA) (Sigma Chemicals, St. Louis), sodium salicylate (Aldrick Chemical Company, Montreal), acetaminophen (Sigma) and phenacetin (Sigma). Concentrations tested ranged from levels considered therapeutic in vivo to those clearly recognized as toxic. Results were analysed by Student's *t*-test and P values equal to or less than 0.05 were considered significant.

## Results

### *PMNL viability*

No reduction in PMNL viability was noted with any of the drugs tested at any concentration or at an ambient temperature of 40°C. In all cases, more than 95% of cells were viable after 3 hours of incubation.

### *Chemotactic migration*

A temperature of 40°C and concentrations of ASA and sodium salicylate up to 50 mg/dL had no effect on PMNL migration. However, activity was suppressed with sodium salicylate at a concentration of 75 ng/dL. Acetaminophen in concentrations up to 100 μg/mL did not affect migration whereas phenacetin at all concentrations tested (20 to 100 μg/mL) did (Fig. 1). Because salicylates reduce PMNL adhesion,[10] cell counts were made from the fluid of the lower compartment of the Boyden chambers of drug-treated and nontreated cells. In all cases a concentration of PMNL of less than $2.0 \times 10^8$/L was found, ruling out the possibility that the low values observed with sodium salicylate and phenacetin were secondary to impaired adhesion of the cells to the undersurface of the chemotactic filters.

### *Bactericidal activity*

Both ASA and sodium salicylate were associated with reduced staphylococcidal activity at concentrations of 25 mg/dL while acetaminophen at a concentration of 100 μg/mL was not. Phenacetin caused marked depression of bactericidal activity at this concentration. Shortly after bacterial–PMNL incubation, an ambient temperature of 40°C favoured staphylococcidal activity. After 2 hours of incubation the situation was reversed (Fig. 2).

## Discussion

Whether fever is detrimental to the course of an infectious illness in humans is unknown. The associated tachycardia, increased cardiac output and negative nitrogen balance[11] can be so viewed, particularly in the elderly or chronically ill. During childhood, convulsions may be associated with fever although it is believed that the rapidity of temperature change, rather than the temperature itself, is the main cause (C.G. Ray: personal communication, 1976). Conversely, in certain experimentally induced viral[12] and bacterial[13] infections, euthermia may result in an increased mortality. Further it has been shown that antipyretics can increase the shedding of rhinoviruses by humans with upper respiratory tract infections.[14]

Antipyretic agents often have undesirable side effects. Platelet function may be abnormal at low blood salicylate concentrations, while at concentrations greater than 25 mg/dL, acid–base, neurologic and renal abnormalities occur.[4] Phenacetin is used infrequently at present because of its well recognized association with renal damage.[7] In vivo phenacetin is altered by the liver to acetaminophen,[15] a compound that appears relatively nontoxic, but which in large amounts can cause liver injury and sometimes death.[5]

None of the drugs tested adversely affected PMNL viability after 3 hours. ASA and acetaminophen had no effect on chemotactically mediated PMNL migration although phenacetin and high concentrations of sodium salicylate did. In vivo ASA is reduced to the salicylate salt.[4] Therefore, the results obtained with sodium salicylate may be more relevant than those obtained with ASA. Both salicylates at a concentration of 25 mg/dL, which in the blood is generally considered nontoxic, suppressed PMNL bactericidal activity. Phenacetin exerted a similar effect whereas acetaminophen at concentrations up to 100 μg/dL did not.

Salicylates alter leukocyte metabolism and function. With lymphocytes, concentrations of 30 mg/dL suppress nucleic acid and protein synthesis.[16] In addition, salicylates suppress the

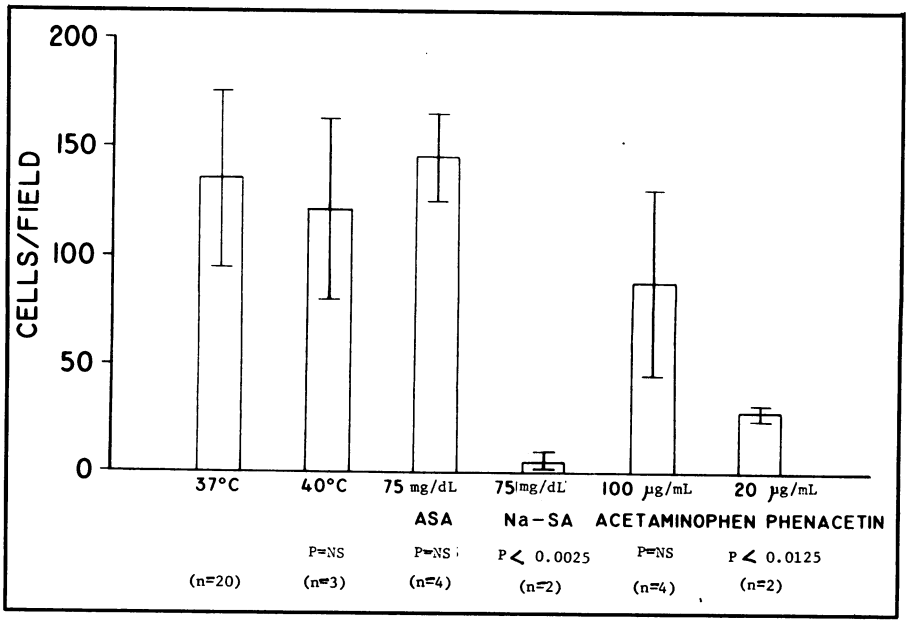


**FIG. 1—Polymorphonuclear leukocyte (PMNL) chemotactic activity. Ambient temperature of 40°C and acetylsalicylic acid (ASA) and acetaminophen did not adversely affect PMNL migration, whereas sodium salicylate and phenacetin did. Results represent mean ± standard deviation. Na–SA = sodium salicylate; NS = not significant.**

generation of hydrogen peroxide within phagocytosing PMNLs.[17] ASA and acetaminophen appear to have no effect on the accumulation of leukocytes in avian joints following injection of sodium urate.[18] This finding is consistent with our observation that these agents at therapeutic blood concentrations have no effect on PMNL migration. The reduction in the generation of hydrogen peroxide, as well as Pachman's[17] demonstration of normal bacterial phagocytosis in the presence of salicylate, is consistent with the possibility that the reduced bactericidal activity in our study was secondary to impaired intracellular killing. The mechanism may be interference with lysosomal lability.[19]

Evidence to date indicates clearly that salicylates and acetaminophen act centrally to control fever[20] rather than through interference with the production or release of leukocyte endogenous pyrogen. It therefore seems unlikely that interference with PMNL metabolism is necessary to produce the antipyretic effect of these agents.

In view of the similarities in PMNL migratory and bactericidal activity at 37° and 40°C, one might question whether hyperpyrexia need be controlled in patients with acute bacterial infections. As acetaminophen has no demonstrable adverse effect on these PMNL functions at concentrations well in excess of those seen clinically[21] and is as effective an antipyretic as ASA,[22,23] it may be the preferred drug, if one is used.

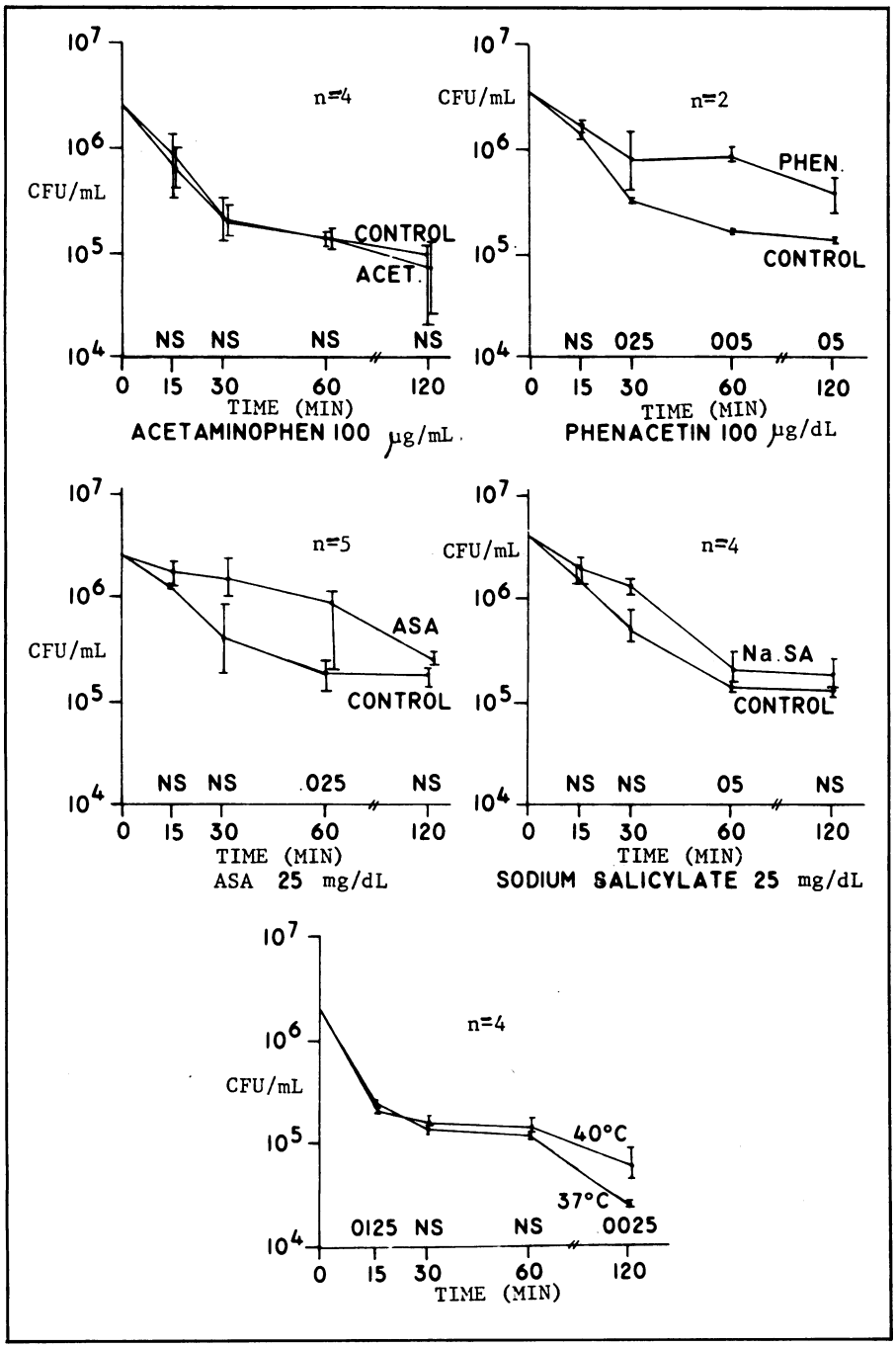


**FIG. 2—Polymorphonuclear leukocyte bactericidal activity. Acetaminophen and ambient temperature of 40°C did not adversely affect bactericidal activity whereas phenacetin, ASA and sodium salicylate did. At concentrations of 50 and 75 mg/dL salicylates were associated with greater suppression of staphylococcidal activity. Results represent mean ± standard deviation. P values are shown along ordinate. CFU = colony forming units.**

## References


1. KLASTERSKY J, KASS EH: Is suppression of fever or hypothermia useful in experimental and clinical infectious diseases? *J Infect Dis* 121: 81, 1970
2. Idem: The effect of suppression of fever upon mortality rate in experimental pneumococcal sepsis. *Clin Res* 17: 370, 1969
3. KLASTERSKY J, CAPPEL R, DEBUSSCHER L: Effectiveness of betamethasone in management of severe infections. A double-blind study. *N Engl J Med* 284: 1248, 1971
4. Antipyretic analgesics, in *Review of Medical Pharmacology*, 3rd ed, MEYERS FH (ed), Los Altos, Lange, 1972, pp 265-75
5. TOGHILL PJ, WILLIAMS R, STEPHENS JD, et al: Acute hepatic necrosis following an overdose of paracetamol. *Gastroenterology* 56: 773, 1969
6. FORDHAM CC, HUFFINES WD, WELT LG: Phenacetin-induced renal lesions in the rat, in *Progress in Pyelonephritis*, KASS EH (ed), Philadelphia, Davis, 1965, p 325
7. PINCUS SH, KLEBANOFF SJ: Quantitative leukocyte iodination. *N Engl J Med* 284: 744, 1971
8. BOYDEN S: The chemotactic effect of mixtures of antibody and antigen on polymorphonuclear leukocytes. *J Exp Med* 115: 453, 1962
9. HARRIS MB, DJERMASSI I, SCHWARTZ E, et al: Polymorphonuclear leukocytes prepared by continuous flow filtration leukapheresis: viability and function. *Blood* 44: 707, 1974
10. MACGREGOR RR, SPAGNUOLO PJ, LENTNEK AL: Inhibition of granulocyte adherence by ethanol, prednisone, and aspirin, measured with an assay system. *N Engl J Med* 291: 642, 1974
11. PETERSDORF R: Chills and fever, in *Harrison's Principles of Internal Medicine*, 7th ed, WINTROBE MM, THORN GW, ADAMS RD, et al (eds), New York, McGraw, 1974, p 54
12. KILBOURNE ED, HORSFALL FL JR: Lethal infection with coxsackie virus in adult mice given cortisone. *Proc Soc Exp Biol Med* 77: 135, 1951
13. BERNHEIM HA, KLUGER MJ: Fever: effect of drug-induced antipyreses on survival. *Science* 193: 237, 1976
14. STANLEY ED, JACKSON GG, PANUSARN C, et al: Increased virus shedding with aspirin treatment of rhinovirus infection. *JAMA* 231: 1248, 1975
15. BRODIE BB, AXELROD J: The fate of acetophenetidin (phenacetin) in man and methods for the estimation of acetophenetidin and its metabolites in biological material. *J Pharmacol Exp Ther* 97: 58, 1949
16. PACHMAN LM, ESTERLY NB, PETERSON RDA: The effect of salicylate on the metabolism of normal and stimulated human lymphocytes in vitro. *J Clin Invest* 50: 226, 1971
17. PACHMAN LM: The effect of salicylate on the function and metabolism of peripheral blood leukocytes (abstr). *Fed Proc* 29: 492, 1970
18. BRUNE K, GLATT M: The avian microcrystal arthritis. IV. The impact of sodium salicylate, acetaminophen and colchicine on leukocyte invasion and enzyme liberation in vivo. *Agents Actions* 4: 101, 1974
19. MILLER WS, SMITH JG JR: The effect of acetylsalicylic acid on lysosomes. *Proc Soc Exp Biol Med* 122: 634, 1966
20. CLARK WG, ALDERDICE MT: Inhibition of leukocytic pyrogen-induced fever by intracerebroventricular administration of salicylate and acetaminophen in the cat. *Proc Soc Exp Biol Med* 140: 399, 1972
21. GWILT JR, ROBERTSON A, MCCHESNEY EW: Determination of blood and other tissue concentrations of paracetamol in dog and man. *J Pharm Pharmacol* 15: 440, 1963
22. BEAVER WT: Mild analgesics. A review of their clinical pharmacology. *Am J Med Sci* 250: 577, 1965
23. Idem: Mild analgesics. A review of their clinical pharmacology. II. *Am J Med Sci* 251: 576, 1966